

Department of Clinical Laboratory Sciences

Graduate Masters Theses

Quantitation Of Amlodipine, Nifedipine, Lisinopril, Atenolol And Hydrochlorothiazide In Postmortem Human Samples Using Liquid Chromatography-With Electrospray Tandem Mass Spectrometry Detection. Myrna Morales, MS. May 2009.

ABSTRACT:

A reversed phase high pressure liquid chromatography with tandem mass spectrometry method using electrospray ionization (LC/MS/MS-EI) was developed for the determination of five anti-hypertensive drugs: amlodipine, nifedipine, atenolol, lisinopril and hydrochlorothiazide in post-mortem samples. The five drugs were extracted from whole blood samples using an 85:15 acetonitrile:methanol extraction solvent for protein precipitation. Analyte separation was achieved with a Luna C18 column (5 μ m 2.0 i.d. x 50 mm) and a linear gradient with a mobile phase mixture consisting of 5mM ammonium acetate with 0.1% formic acid and 0.1% formic acid in acetonitrile. The mode of detection used both positive and negative electrospray ionization with multiple reaction monitoring. Calibration curves were linear with correlation coefficients (r) greater than 0.99 and correlations of determination (r^2) greater than or equal to 0.99. Between-day and within-day precision and accuracy (%Bias) study results ranged from 0.1% - 16.1% CV and 0.2 to 25.2 % bias, respectively. The high bias observed was due to ion suppression between lisinopril and hydrochlorothiazide at the lower and upper levels of quantitation. Stability studies showed that samples were not stable when stored at room temperature. Post-mortem samples were successfully quantitated with the method. The method can easily be incorporated into a forensic laboratory because it will simultaneously analyze for the five drugs after being extracted via protein precipitation, a very low cost sample clean up procedure, enhancing the laboratory's analytical capability. The method allows for the detection and quantitation of five anti-hypertensive drugs that are not currently being analyzed for in many forensic laboratories and can easily be incorporated in other laboratories, for therapeutic drug monitoring or clinical research.